Amendments to the Specification

Please amend the specification as follows:

Please amend the section beginning on page 5, line 10, and continuing through line 13 as follows:

The invention further relates to an isolated nucleic acid molecule ~~of~~ of SEQ ID NO:1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1 or the cDNA sequence included in the deposited clone, which is hybridizable to SEQ ID NO:1.

Please amend the section beginning on page 5, line 31, and continuing through line 33 as follows:

The invention further relates to the isolated polypeptide of ~~of~~ SEQ ID NO:2, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

Please amend the section beginning on page 6, line 14, and continuing through line 19 as follows:

The invention further relates to a method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising the steps of (a) determining the presence or amount of expression of the polypeptide of ~~of~~ SEQ ID NO:2 in a biological sample; and diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

Please amend the section beginning on page 10, line 1, and continuing through line 23 as follows:

Figures 2A-B show the regions of similarity of K+betaM2 to other electrically silent beta subunit, specifically, the human potassium channel K+Hnov28 protein (Genbank Accession No. gi|Y34129; SEQ ID NO:4), the Drosophila CG10830 protein (Genbank Accession No. gi|AAF55820.1; SEQ ID NO:5), the Caenorhabditis K+ channel tetramerisation domain (Genbank Accession No. gi|CAA20329.1; SEQ ID NO:6), and the human potassium channel K+Hnov27 (Genbank Accession No. gi|Y34125; SEQ ID NO:7). The alignment was performed ~~perfomed~~ using the CLUSTALW algorithm described elsewhere herein. The darkly shaded amino acids represent

regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Lines between residues indicate gapped regions for the aligned polypeptides.

Figure 3 shows an expression profile of the novel human potassium channel beta-subunit, K+betaM2. The figure illustrates the relative expression level of K+betaM2 amongst various mRNA tissue sources. As shown, transcripts corresponding to K+betaM2 expressed highly in testis. The K+betaM2 polypeptide was also expressed significantly in pancreas, brain, and to a lesser extent, in lung and spinal cord. Expression data was obtained by measuring the steady state K+betaM2 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NOs:9 and 10 ~~SEQ ID NO:8 and 9~~ as described herein.

Please amend the section beginning on page 11, line 5, and continuing through line 14 as follows:

Figure 5 shows an expanded expression profile of the novel full-length human methionine aminopeptidase K+betaM2 protein. The figure illustrates the relative expression level of K+betaM2 amongst various mRNA tissue sources. As shown, the K+betaM2 polypeptide was expressed predominately in the brain, with the highest expression in the cortex followed by the hippocampus, nucleus accumbens, caudate, amygdala and hypothalamus. K+betaM2 was also significantly expressed in the thyroid gland, the pituitary gland, the pineal gland and the dorsal root ganglia. Expression data was obtained by measuring the steady state K+betaM2 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NOs:22 and 23~~SEQ ID NO:21 and 22~~, and Taqman probe (SEQ ID NO:24~~23~~) as described in Example 5 herein.

Please amend the section beginning on page 13, line 18, and continuing through line 33 as follows:

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer ~~sequnencer~~ (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted ~~pridcted~~ by translation of a DNA sequence determined above. Therefore, as is known in the art for any DNA sequence ~~seuqnece~~ determined ~~detemrined~~ by this automated approach, any nucleotide sequence ~~seqence~~ determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about

90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence ~~seqnece~~ of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined ~~detemrined~~ nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by ~~bt~~ the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Please amend the section beginning on page 13, line 34, and continuing through line 7 of page 14 as follows:

Using the information provided herein, such as the nucleotide ~~nucletide~~ sequence in Figures 1A-D (SEQ ID NO:1), a nucleic acid molecule of the present invention encoding the K+betaM2 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figures 1A-D (SEQ ID NO:1) was discovered in a cDNA library derived from human brain.

Please amend the section beginning on page 14, line 21, and continuing through line 32 as follows:

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH2PO4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg~~ug~~/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Please amend the section beginning on page 17, line 6, and continuing through line 8 as follows:

The term "organism" as referred to herein is meant to encompass any organism referenced herein, though preferably to eukaryotic organisms ~~organsisms~~, more preferably to mammals, and most preferably to humans.

Please amend the section beginning on page 17, line 9, and continuing through line 14 as follows:

The present invention encompasses the identification of proteins, nucleic acids, or other molecules, that bind to polypeptides and polynucleotides of the present invention (for example, in a receptor-ligand interaction). The polynucleotides of the present invention can also be used in interaction trap assays (such as, for example, that described ~~discribed~~ by Ozenberger and Young (Mol Endocrinol., 9(10):1321-9, (1995); and Ann. N. Y. Acad. Sci., 7;766:279-81, (1995)).

Please amend the section beginning on page 17, line 15, and continuing through line 21 as follows:

The polynucleotide and polypeptides of the present invention are useful as probes for the identification and isolation of full-length cDNAs and/or genomic DNA which correspond to the polynucleotides of the present invention, as probes to hybridize and discover novel, related DNA sequences, as probes for positional cloning of this or a related sequence, as probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides, as probes to quantify gene expression, and as probes for microarrays ~~microarays~~.

Please amend the section beginning on page 17, line 24, through line 26 as follows:

Also, in preferred embodiments the present invention provides methods for further refining the biological function ~~fuction~~ of the polynucleotides and/or polypeptides of the present invention.

Please amend the section beginning on page 18, line 35, and continuing through page 19, line 10 as follows:

The K+betaM2 polypeptide was determined to share 31% identity and 41% similarity with the ~~the~~ human potassium channel K+Hnov28 protein (Genbank Accession No. gi|Y34129; SEQ ID NO:4); 52% identity and 66% similarity with the Drosophila CG10830 protein (Genbank Accession

No. gi|AAF55820.1; SEQ ID NO:5); 42% identity and 51% similarity with the Caenorhabditis K+ channel tetramerisation domain (Genbank Accession No. gi|CAA20329.1; SEQ ID NO:6); and 32% identity and 41% similarity with the human potassium channel K+Hnov27 (Genbank Accession No. gi|Y34125; SEQ ID NO:7).

Please amend the section beginning on page 19, line 26, and continuing through page 20, line 12 as follows:

Expanded analysis of K+betaM2 expression levels by TaqMan™ quantitative PCR (see Figure 5) confirmed and extended the earlier results obtained with SYBR green. K+betaM2 mRNA was predominantly ~~predoimately~~ expressed in the brain, and specifically in the following brain sub-regions: cortex, followed by the hippocampus, nucleus accumbens, caudate, amygdala and hypothalamus. Expression in the cerebellum, medulla oblongata, substantia nigra was limited. These data suggest a role for K+betaM2 that is involved primarily in higher brain functions of learning, memory and emotions and other disorders described herein, and less relative to the control of movement and cardiorespiratory reflex integration. The SYBR green (Figure 4) experiments indicated low level expression in the kidney. The TaqMan™ results (Figure 5) indicated K+betaM2 expression was restricted to the medulla and cortex of the kidney, and was virtually absent from the pelvis and the renal blood vessel. Expression in the pancreas was not observed using TaqMan™ analysis. Of particular interest is the newly appreciated K+betaM2 expression in the thyroid gland, the pituitary gland, the pineal gland and the dorsal root ganglia, as revealed by TaqMan™ using an extended panel of tissue RNAs. These data suggest that K+betaM2 may also have a role in the control of neuroendocrine regulation of thyroid hormone release and pain transmission and may be a drug target for the treatment of various metabolic disorders under the influence of thyroid hormones.

Please amend the section beginning on page 20, line 35, and continuing through page 21, line 14 as follows:

There are a number of potassium channel subtypes. Physiologically, one important subtype is the maxi-K channel, defined as high -conductance calcium-activated potassium channel, which is present in neuronal tissue and smooth muscle. Intracellular calcium concentration (Ca^{2+}_i ~~Ca.sup.2+.sub.i~~) and membrane potential gate these channels. For example, maxi-K channels are opened to enable efflux of potassium ions by an increase in the intracellular Ca^{2+}~~Ca.sub.2+~~ concentration or by membrane depolarization (change in potential). Elevation of intracellular

calcium concentration is required for neurotransmitter release, smooth muscle contraction, proliferation of some cell types and other processes. Modulation of maxi-K channel activity therefore affects cellular processes that depend on influx of calcium through voltage-dependent pathways, such as transmitter release from the nerve terminals and smooth muscle contraction.

Please amend the section beginning on page 24, line 5, and continuing through line 19 as follows:

In addition, the strong homology to human potassium channel beta subunits, combined with the localized expression in kidney tissue suggests the K+betaM2 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing renal diseases and/or disorders, which include, but are not limited to: nephritis, renal failure, nephrotic syndrome, urinary tract infection, hematuria, proteinuria, oliguria, polyuria, nocturia, edema, hypertension, electrolyte disorders, sterile pyuria, renal osteodystrophy, large kidneys, renal transport defects, nephrolithiasis, azotemia, anuria, urinary retention ,slowing of urinary stream, large prostate, flank tenderness, full bladder sensation after voiding, enuresis, dysuria,bacteriuria, kidney ~~kideny~~ stones, glomerulonephritis, vasculitis, hemolytic uremic syndromes, thrombotic thrombocytopenic purpura, malignant hypertension, casts, tubulointerstitial kidney diseases, renal tubular acidosis, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, and/or renal colic, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome.for example.

Please amend the section beginning on page 24, line 20, and continuing through line 26 as follows:

Alternatively, the strong homology to potassium channel beta subunits combined with the expression in encodrine ~~enderine~~ tissues suggests the K+betaM2 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing disorders related to the thyroid gland, the pituitary gland, the pineal gland, or the dorsal root ganglia, disorders related to aberrant neuroendocrine regulation of thyroid hormone release, pain, and/or metabolic disorders related to aberrant ~~abberrant~~ thyroid hormone production or secretion.

Please amend the section beginning on page 24, line 27, and continuing through page 25, line 7, as follows:

Moreover, K+betaM2 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing disorders the following endocrine disorders, which include, but are not limited to, the following: aberrant growth hormone synthesis and/or secretion, aberrant prolactin synthesis and/or secretion, aberrant luteinizing hormone synthesis and/or secretion, aberrant follicle-stimulating hormone synthesis and/or secretion, aberrant thyroid-stimulating hormone synthesis and/or secretion, aberrant adrenocorticotropin synthesis and/or secretion, aberrant vasopressin secretion, aberrant oxytocin secretion, aberrant growth, aberrant lactation, aberrant sexual characteristic development, aberrant testosterone synthesis and/or secretion, aberrant estrogen synthesis and/or secretion, aberrant water homeostasis, hypogonadism, Addison's disease, hypothyroidism, Cushing's disease, agromegaly, gigantism, lethargy, osteoporosis, aberrant calcium homeostasis, aberrant potassium homeostasis, reproductive disorders, and/or developmental disorders ~~disoders~~.

Please amend the section beginning on page 25, line 19, and continuing through line 23 as follows:

Alternatively, K+betaM2 polypeptides of the invention, or agonists thereof, are administered to treat, prevent, prognose, and/or diagnose disorders involving excessive smooth muscle tone or excitability, which include, but are not limited to asthma, angina, hypertension, incontinence, pre-term labor, and irritable ~~irratible~~ bowel syndrome.

Please amend the section beginning on page 28, line 20, and continuing through line 29 as follows:

In addition, the function of the protein may be assessed by applying quantitative PCR methodology, for example. Real time quantitative PCR would provide the capability of following the expression of the K+betaM2 gene throughout development, for example. Quantitative PCR methodology requires only a nominal amount of tissue from each developmentally important step is needed to perform such experiments ~~experiements~~. Therefore, the application of quantitative PCR methodology to refining the biological function of this polypeptide is encompassed by the present invention. Also encompassed by the present invention are quantitative PCR probes corresponding to the polynucleotide sequence provided as SEQ ID NO:1 (Figures 1A-D).

Please amend the section beginning on page 28, line 30, and continuing through line 36 as follows:

The function of the protein may also be assessed through complementation assays in yeast. For example, in the case of the K+betaM2, transforming yeast deficient in potassium channel beta subunit activity and assessing their ability to grow would provide convincing evidence the K+betaM2 polypeptide has potassium channel beta subunit activity. Additional assay conditions and methods that may be used in assessing the function of the polynucleotides ~~polynucletides~~ and polypeptides of the present invention are known in the art, some of which are disclosed elsewhere herein.

Please amend the section beginning on page 29, line 8, and continuing through line 19 as follows:

Moreover, the biological function of this polypeptide may be determined by the application of antisense and/or sense methodology and the resulting generation of transgenic mice and/or rats. Expressing a particular gene in either sense or antisense orientation in a transgenic mouse or rat could lead to respectively higher or lower expression levels of that particular gene. Altering the endogenous expression levels of a gene can lead to the observation ~~obervation~~ of a particular phenotype that can then be used to derive indications on the function of the gene. The gene can be either over-expressed or under expressed in every cell of the organism at all times using a strong ubiquitous promoter, or it could be expressed in one or more discrete parts of the organism using a well characterized tissue-specific promoter (e.g., a testis, pancreas, brain, kidney, or spinal cord specific promoter), or it can be expressed at a specified time of development using an inducible and/or a developmentally regulated promoter.

Please amend the section beginning on page 37, line 16, and continuing through line 22 as follows:

In preferred embodiments, the present invention encompasses a polynucleotide lacking the initiating start codon, in addition to, the resulting encoded polypeptide of K+betaM2. Specifically, the present invention encompasses the polynucleotide corresponding to nucleotides 518 through ~~thru~~ 1798 of SEQ ID NO:1, and the polypeptide corresponding to amino acids 2 through ~~thru~~ 428 of SEQ ID NO:2. Also encompassed are recombinant vectors comprising said encoding sequence, and host cells comprising said vector.

Please amend the section beginning on page 41, line 30, and continuing through page 42, line 6, as follows:

The present invention also encompasses polynucleotides capable of hybridizing, preferably under reduced stringency conditions, more preferably under stringent conditions, and most preferably under highly stringent ~~stingent~~ conditions, to polynucleotides described herein. Examples of stringency conditions are shown in Table II below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Please amend the section beginning on page 44, line 5, and continuing through line 14 as follows:

‡ - The "hybrid length" is the anticipated length for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide ~~polynucletotide~~ of unknown sequence, the hybrid is assumed to be that of the hybridizing polynucleotide of the present invention. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity. Methods of aligning two or more polynucleotide sequences and/or determining the percent identity between two polynucleotide sequences are well known in the art (e.g., MegAlign program of the DNA*Star suite of programs, etc).

Please amend the section beginning on page 44, line 14, and continuing through line 19 as follows:

† - SSPE (1xSSPE is 0.15M NaCl, 10mM NaH2PO4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and ~~annd~~ 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete. The hydridizations and washes may additionally include 5X Denhardt's reagent, .5-1.0% SDS, 100µg~~u~~g/ml denatured, fragmented salmon sperm DNA, 0.5% sodium pyrophosphate, and up to 50% formamide.

Please amend the section beginning on page 47, line 17, and continuing through line 26 as follows:

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:1 and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described ~~desribed~~ below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Please amend the section beginning on page 47, line 29, and continuing through line 36 as follows:

The present invention also encompasses ~~encompases~~ variants (e.g., allelic variants, orthologs, etc.) of the polynucleotide sequence disclosed herein in SEQ ID NO:1, the complementary strand thereto, and/or the cDNA sequence contained in the deposited clone.

The present invention also encompasses variants of the polypeptide sequence, and/or fragments therein, disclosed in SEQ ID NO:2, a polypeptide encoded by the polynucleotide ~~polunucleotide~~ sequence in SEQ ID NO:1, and/or a polypeptide encoded by a cDNA in the deposited clone.

Please amend the section beginning on page 48, line 9, and continuing through line 36 as follows:

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a K+betaM2 related polypeptide having an amino acid sequence as shown in the sequence listing and described in SEQ ID NO:1 or the cDNA contained in ATCC deposit No:PTA-2966; (b) a nucleotide sequence encoding a mature K+betaM2 related polypeptide having the amino acid sequence as shown in the sequence listing and described in SEQ ID NO:1 or the cDNA contained in ATCC deposit No:PTA-2966; (c) a nucleotide sequence encoding a biologically active fragment of a K+betaM2 related polypeptide having an amino acid sequence shown in the sequence listing and described in SEQ ID NO:1 or the cDNA contained in

ATCC deposit No:PTA-2966; (d) a nucleotide sequence encoding an antigenic fragment of a K+betaM2 related polypeptide having an amino acid sequence shown in the sequence listing and described in SEQ ID NO:1 or the cDNA contained in ATCC deposit No:PTA-2966; (e) a nucleotide sequence encoding a K+betaM2 related polypeptide comprising the complete amino acid sequence encoded by a human cDNA plasmid contained ~~containined~~ in SEQ ID NO:1 or the cDNA contained in ATCC deposit No:PTA-2966; (f) a nucleotide sequence encoding a mature K+betaM2 related ~~realted~~ polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:1 or the cDNA contained in ATCC deposit No:PTA-2966; (g) a nucleotide sequence encoding a biologically active fragment ~~fragement~~ of a K+betaM2 related polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:1 or the cDNA contained in ATCC deposit No:PTA-2966; (h) a nucleotide sequence encoding an antigenic fragment of a K+betaM2 related polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:1 or the cDNA contained in ATCC deposit No:PTA-2966; (I) a nucleotide sequence complimentary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above.

Please amend the section beginning on page 51, line 21, and continuing through page 52, line 14, as follows:

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the CLUSTALW computer program (Thompson, J.D., et al., Nucleic Acids Research, 2(22):4673-4680, (1994)), which is based on the algorithm of Higgins, D.G., et al., Computer Applications in the Biosciences (CABIOS), 8(2):189-191, (1992). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. However, the CLUSTALW algorithm automatically converts U's to T's when comparing RNA sequences to DNA sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a CLUSTALW alignment of DNA sequences to calculate percent identity via pairwise alignments are: Matrix=IUB, k-tuple=1, Number of Top Diagonals=5, Gap

Penalty=3, Gap Open Penalty 10, Gap Extension Penalty=0.1, Scoring Method=Percent, Window Size=5 or the length of the subject nucleotide sequence, whichever is shorter. For multiple alignments, the following CLUSTALW parameters are preferred: Gap Opening Penalty=10; Gap Extension Parameter=0.05; Gap Separation Penalty Range=8; End Gap Separation Penalty=Off; % Identity for Alignment Delay=40%; Residue Specific Gaps:Off; Hydrophilic Residue Gap=Off; and Transition Weighting=0. The pairwise and multiple ~~multple~~ alignment parameters provided for CLUSTALW above represent the default parameters as provided with the AlignX software program (Vector NTI suite of programs, version 6.0).

Please amend the section beginning on page 53, line 34, and continuing through page 54, line 25, as follows:

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% identical to, for instance, an amino acid sequence referenced in Table 1 (SEQ ID NO:2) or to the amino acid sequence encoded by cDNA contained in a deposited clone, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the CLUSTALW computer program (Thompson, J.D., et al., Nucleic Acids Research, 2(22):4673-4680, (1994)), which is based on the algorithm of Higgins, D.G., et al., Computer Applications in the Biosciences (CABIOS), 8(2):189-191, (1992). In a sequence alignment the query and subject sequences are both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a CLUSTALW alignment of DNA sequences to calculate percent identity via pairwise alignments are: Matrix=BLOSUM, k-tuple=1, Number of Top Diagonals=5, Gap Penalty=3, Gap Open Penalty 10, Gap Extension Penalty=0.1, Scoring Method=Percent, Window Size=5 or the length of the subject nucleotide sequence, whichever is shorter. For multiple alignments, the following CLUSTALW parameters are preferred: Gap Opening Penalty=10; Gap Extension Parameter=0.05; Gap Separation Penalty Range=8; End Gap Separation Penalty=Off; % Identity for Alignment Delay=40%; Residue Specific Gaps:Off; Hydrophilic Residue Gap=Off; and Transition Weighting=0. The pairwise and multiple ~~multple~~ alignment parameters provided for CLUSTALW above represent the default parameters as provided with the AlignX software program (Vector NTI suite of programs, version 6.0).

Please amend the section beginning on page 55, line 35, and continuing through page 56, line 12, as follows:

In addition to the above method of aligning two or more polynucleotide or polypeptide sequences to arrive at a percent identity value for the aligned sequences, it may be desirable in some circumstances to use a modified version of the CLUSTALW algorithm which takes into account known structural features of the sequences to be aligned, such as for example, the SWISS-PROT designations for each sequence. The result of such a modified ~~modifed~~ CLUSTALW algorithm may provide a more accurate value of the percent identity for two polynucleotide or polypeptide sequences. Support for such a modified version of CLUSTALW is provided within the CLUSTALW algorithm and would be readily appreciated to one of skill in the art of bioinformatics.

Please amend the section beginning on page 77, line 24, and continuing through page 78, line 14, as follows:

The skilled artisan would acknowledge that a variety of methods exist in the art for the production of monoclonal antibodies and thus, the invention is not limited to their sole production in hydridomas. For example, the monoclonal antibodies may be made by recombinant DNA methods, such as those described in US patent No. 4, 816, 567. In this context, the term "monoclonal antibody" refers to an antibody derived from a single eukaryotic, phage, or prokaryotic clone. The DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies, or such chains from human, humanized, or other sources). The hybridoma ~~hydridoma~~ cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transformed into host cells such as Simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (US Patent No. 4, 816, 567; Morrison et al, supra) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for

the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

Please amend the section beginning on page 98, line 26, and continuing through page 99, line 6, as follows:

Moreover, MIPs based upon the structure of the polypeptide(s) of the present invention may be useful in screening for compounds that bind to the polypeptide(s) of the invention. Such a MIP would serve the role of a synthetic "receptor" by mimicking ~~minimicking~~ the native architecture of the polypeptide. In fact, the ability of a MIP to serve the role of a synthetic receptor has already been demonstrated for the estrogen receptor (Ye, L., Yu, Y., Mosbach, K, Analyst., 126(6):760-5, (2001); Dickert, F, L., Hayden, O., Halikias, K, P, Analyst., 126(6):766-71, (2001)). A synthetic receptor may either be mimicked in its entirety (e.g., as the entire protein), or mimicked as a series of short peptides corresponding to the protein (Rachkov, A., Minoura, N, Biochim, Biophys, Acta., 1544(1-2):255-66, (2001)). Such a synthetic receptor MIPs may be employed in any one or more of the screening methods described elsewhere herein.

Please amend the section beginning on page 121, line 30, and continuing through page 122, line 12, as follows:

Moreover, the polypeptides of the present invention can be fused to marker sequences (also referred to as "tags"). Due to the availability of antibodies specific to such "tags", purification of the fused polypeptide of the invention, and/or its identification is significantly facilitated since antibodies specific to the polypeptides of the invention are not required. Such purification may be in the form of an affinity purification whereby an anti-tag antibody or another type of affinity matrix (e.g., anti-tag antibody attached to the matrix of a flow-through ~~thru~~ column) that binds to the epitope tag is present. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984)).

Please amend the section beginning on page 122, line 13, and continuing through line 25 as follows:

The skilled artisan would acknowledge the existence of other "tags" which could be readily substituted for the tags referred to supra for purification and/or identification of polypeptides of the present invention (Jones C., et al., J Chromatogr A. 707(1):3-22 (1995)). For example, the c-myc tag and the 8F9, 3C7, 6E10, G4m B7 and 9E10 antibodies thereto (Evan et al., Molecular and Cellular Biology 5:3610-3616 (1985)); the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky et al., Protein Engineering, 3(6):547-553 (1990), the Flag–peptide – i.e., the octapeptide sequence DYKDDDDK (SEQ ID NO:26~~25~~), (Hopp et al., Biotech. 6:1204-1210 (1988); the KT3 epitope peptide (Martin et al., Science, 255:192-194 (1992)); a-tubulin epitope peptide (Skinner et al., J. Biol. Chem..., 266:15136-15166, (1991)); the T7 gene 10 protein peptide tag (Lutz-Freyermuth et al., Proc. Natl. Sci. USA, 87:6363-6397 (1990)), the FITC epitope (Zymed, Inc.), the GFP epitope (Zymed, Inc.), and the Rhodamine epitope (Zymed, Inc.).

Please amend the section beginning on page 126, line 34, and continuing through page 127, line 8, as follows:

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a Pichia ~~Pichea~~ yeast system essentially as described in "Pichia Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong AOX1 promoter linked to the Pichia pastoris alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Please amend the section beginning on page 141, line 5, and continuing through line 28 as follows:

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not

present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the stronger binding characteristics of PNA:DNA hybrids. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T_m ~~T.sub.m~~) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

Please amend the section beginning on page 150, line 18 and continuing through line 25 as follows:

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidylcholine ~~phosphatidyl, choline~~, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

Please amend the section beginning on page 187, line 35, and continuing through page 188, line 17 as follows:

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans

(e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat ~~totreat~~, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Please amend the section beginning on page 204, line 15, and continuing through line 25 as follows:

Another aspect of the present invention embraces a method of screening for a compound that is capable of modulating the biological activity of a K+betaM2 polypeptide, comprising providing a host cell containing an expression vector harboring a nucleic acid sequence encoding a K+betaM2 polypeptide, or a functional peptide or portion thereof (e.g., SEQ ID NO ~~NOS~~:2); determining the biological activity of the expressed K+betaM2 polypeptide in the absence of a modulator compound; contacting the cell with the modulator compound and determining the biological activity of the expressed K+betaM2 polypeptide in the presence of the modulator compound. In such a method, a difference between the activity of the K+betaM2 polypeptide in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

Please amend the section beginning on page 223, line 13, and continuing through line 15 as follows:

Salians, M., Duprat, F., Heurteaux, C., Hugnot, J. P., and Lazdunski, M. (1997). New modulatory alpha ~~aplha~~ subunits for mammalian Shab K+ channels. J. Biol. Chem... *272*, 24371-24379.

Please amend the section beginning on page 223, line 28, and extending through page 224, line 10, as follows:

Ion channel sequences were used as probes to search the human genomic sequence database. The search program used was gapped BLAST (Altschul et al., 1997). Ion channel specific Hidden

Markov Models (HMMs) built in-house or obtained from the public PFAM databases were also used as probes (Bateman et al., 2000). The search program used for HMMs was the Genewise/Wise2 package ~~(http://www.sanger.ac.uk/Software/Wise2/index.shtml)~~ . The top genomic exon hits from the results were searched back against the non-redundant protein and patent sequence databases. From this analysis BAC AC008652 was determined to possess a novel ion channel exon based on its homology to the putative human beta subunit K+Hnov28 (SEQ ID NO:4). The full length cDNA described herein as K+betaM2 (SEQ ID NO:1, Figures 1A-D), was isolated using probes designed from the BAC AC008652 exon (SEQ ID NO:3). Based on this analysis, a partial sequence of the novel human ion channel related gene, K+betaM2, was identified directly from the genomic sequence. The full-length clone of this novel ion channel gene was experimentally obtained by using the sequence from genomic data.

Please amend the section beginning on page 224, line 14, and continuing through line 21 as follows:

Brain poly A + RNA was purchased from Clontech and converted into double stranded cDNA using the SuperScript™ Plasmid System for cDNA Synthesis and Plasmid Cloning (Life Technologies) except that no radioisotope was incorporated in either of the cDNA synthesis steps and that the cDNA was fractionated by HPLC. This was accomplished on a TransGenomics HPLC system equipped with a size exclusion column (TosoHass) with dimensions of 7.8mm x 30cm and a particle size of 10μm ~~10□m~~. Tris buffered saline was used as the mobile phase and the column was run at a flow rate of 0.5 mL/min.

Please amend the section beginning on page 229, line 12, and continuing through line 16 as follows:

Exemplary subunits which could be used in the two-hybrid system to assess K+betaM2s ability to associate with other alpha or beta subunits include, but are not limited to, the NH_2 ~~NH.sub.2~~ -terminal domain of the rat Kv9.3, human Shab-related subunit, the human Kv8.1, the Drosophila Shab11 of the Kv2 subfamily, the Shaw2 of the Kv3 subfamily, or the Shal2 of the Kv4 subfamily.

Please amend the section beginning on page 227, line 21, and continuing through line 27 as follows:

For K+betaM2, the primer probe sequences were as follows

Forward Primer	5'- TGGGATTCGGGCTTTGG -3' (SEQ ID NO:2221)
Reverse Primer	5'- TGTTGGGTTGTTACAGACATCATAAA -3' (SEQ ID NO:2322)
TaqMan Probe	5' – TGACACAGCTAAATCCTAGCATGGGCACA -3' (SEQ ID NO:2423)

Please amend the section beginning on page 232, line 17, and extending through line 29 as follows:

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:1 (i.e., within the region of SEQ ID NO:1 bounded by the 5′ NT and the 3′ NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 µl of reaction mixture with 0.5 µg of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl2, 0.01% (w/v) gelatin, 20 µM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Please amend the section beginning on page 236, line 23, and continuing through line 28 as follows:

Tissue Northern blots containing the bound mRNA of various tissues are examined with the labeled probe using ExpressHyb™ hybridization solution (Clonetech according to manufacturers protocol number PT1190-1. Northern blots can be produced using various protocols well known in the art (e.g., Sambrook et al). Following hybridization and washing, the blots are mounted and exposed to film at -70C overnight, and the films developed according to standard procedures.

Please amend the section beginning on page 237, line 30, and continuing through line 36 as follows:

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml). The O/N culture is used to

inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.600) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Please amend the section beginning on page 240, line 7, and continuing through line 12 as follows:

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Coomassie blue stained 16% SDS-PAGE gel when 5 μg of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Please amend the section beginning on page 241, line 15, and continuing through line 22 as follows:

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit (GENECLEAN™, ~~"Geneclean,"~~ BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit (GENECLEAN™, ~~"Geneclean"~~ BIO 101 Inc., La Jolla, Ca.).

Please amend the section beginning on page 241, line 30, and continuing through page 242, line 32 as follows:

Five μg of a plasmid containing the polynucleotide is co-transformed with 1.0 μg of a commercially available linearized baculovirus DNA ("BACULOGOLD™ ~~BaculoGoldtm~~ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One μg of BACULOGOLD™ ~~BaculoGoldtm~~ virus DNA and 5 μg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium are added, mixed and incubated for 15

minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, supra. An agarose gel with BLUE GAL™ ~~"Blue-Gal"~~ (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µ~~u~~l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 µ~~u~~Ci of 35S-methionine and 5 µ~~u~~Ci 35S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Please amend the section beginning on page 244, line 20, and continuing through line 35 as follows:

Chinese hamster ovary cells lacking an active DHFR gene is used for transformation. Five µg of an expression plasmid is cotransformed with 0.5 µ~~u~~g of the plasmid pSVneo using lipofectin (Felgner et al., supra). The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are

trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Please amend the section beginning on page 245, line 30, and continuing through page 246, line 13 as follows:

Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGCCCAG
CACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACC
CTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGTGGTGGACGTAAGCCACGAAGA
CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA
AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCC
TGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT
CCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAG
GTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTG
CCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCT
CTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
GGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:2524)

Please amend the section beginning on page 246, line 23 and continuing through line 34 as follows:

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511

(1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µug/ml of streptomycin.

Please amend the section beginning on page 248, line 26, and continuing through page 249, line 13 as follows:

Following centrifugation, the supernatant is discarded and washed with 75% ethanol. Following ~~Follwing~~ the wash step, the RNA is centrifuged again at 800 rpm for 5 minutes at 4 C. The supernatant is discarded and the pellet allowed to air dry. RNA is the dissolved in DEPC water and heated to 60 C for 10 minutes. Quantities of RNA can be determined using optical density measurements. CDNA may be synthesized, according to methods well-known in the art and/or described herein, from 1. 5-2. 5 micrograms of RNA using reverse transciptase and random hexamer primers. CDNA is then used as a template for PCR amplification of VH and VL domains.

Primers used to amplify VH and VL genes are shown below. Typically a PCR reaction makes use of a single 5'primer and a single 3'primer. Sometimes, when the amount of available RNA template is limiting, or for greater efficiency, groups of 5' and/or 3'primers may be used. For example, sometimes all five VH-5'primers and all JH3'primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0. 7 units of High Fidelity Taq polymerase ~~polymerse~~, 5'primer mix, 3'primer mix and 7. 5 microliters of cDNA. The 5'and 3'primer mix of both VH and VL can be made by pooling together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are : 96 C for 5 minutes ; followed by 25 cycles of 94 C for 1 minute, 50 C for 1 minute, and 72 C for 1 minute ; followed by an extension cycle of 72 C for 10 minutes. After the reaction has been completed, sample tubes may be stored at 4 C.

Please amend the Table beginning on page 249, line 15, and continuing through page 251, line 5 as follows:

Primer Sequences Used to Amplify VH domains.

Primer name	Primer Sequence	SEQ ID NO:
Hu VH1 – 5'	CAGGTGCAGCTGGTGCAGTCTGG	31~~30~~
Hu VH2 – 5'	CAGGTCAACTTAAGGGAGTCTGG	32~~31~~
Hu VH3 – 5'	GAGGTGCAGCTGGTGGAGTCTGG	33~~32~~
Hu VH4 – 5'	CAGGTGCAGCTGCAGGAGTCGGG	34~~33~~
Hu VH5 – 5'	GAGGTGCAGCTGTTGCAGTCTGC	35~~34~~
Hu VH6 – 5'	CAGGTACAGCTGCAGCAGTCAGG	36~~35~~
Hu JH1 – 5'	TGAGGAGACGGTGACCAGGGTGCC	37~~36~~
Hu JH3 – 5'	TGAAGAGACGGTGACCATTGTCCC	38~~37~~
Hu JH4 – 5'	TGAGGAGACGGTGACCAGGGTTCC	39~~38~~
Hu JH6 – 5'	TGAGGAGACGGTGACCGTGGTCCC	40~~39~~

Primer Sequences Used to Amplify VL domains

Primer name	Primer Sequence	SEQ ID NO:
Hu Vkappa1 – 5'	GACATCCAGATGACCCAGTCTCC	41~~40~~
Hu Vkappa2a – 5'	GATGTTGTGATGACTCAGTCTCC	42~~41~~
Hu Vkappa2b – 5'	GATATTGTGATGACTCAGTCTCC	43~~42~~
Hu Vkappa3 – 5'	GAAATTGTGTTGACGCAGTCTCC	44~~43~~
Hu Vkappa4 – 5'	GACATCGTGATGACCCAGTCTCC	45~~44~~
Hu Vkappa5 – 5'	GAAACGACACTCACGCAGTCTCC	46~~45~~
Hu Vkappa6 – 5'	GAAATTGTGCTGACTCAGTCTCC	47~~46~~
Hu Vlambda1 – 5'	CAGTCTGTGTTGACGCAGCCGCC	48~~47~~
Hu Vlambda2 – 5'	CAGTCTGCCCTGACTCAGCCTGC	49~~48~~
Hu Vlambda3 – 5'	TCCTATGTGCTGACTCAGCCACC	50~~49~~
Hu Vlambda3b – 5'	TCTTCTGAGCTGACTCAGGACCC	51~~50~~
Hu Vlambda4 – 5'	CACGTTATACTGACTCAACCGCC	52~~51~~
Hu Vlambda5 – 5'	CAGGCTGTGCTCACTCAGCCGTC	53~~52~~
Hu Vlambda6 – 5'	AATTTTATGCTGACTCAGCCCCA	54~~53~~
Hu Jkappa1 – 3'	ACGTTTGATTTCCACCTTGGTCCC	55~~54~~
Hu Jkappa2 – 3'	ACGTTTGATCTCCAGCTTGGTCCC	56~~55~~
Hu Jkappa3 – 3'	ACGTTTGATATCCACTTTGGTCCC	57~~56~~
Hu Jkappa4 – 3'	ACGTTTGATCTCCACCTTGGTCCC	58~~57~~
Hu Jkappa5 – 3'	ACGTTTAATCTCCAGTCGTGTCCC	59~~58~~
Hu Vlambda1 – 3'	CAGTCTGTGTTGACGCAGCCGCC	60~~59~~
Hu Vlambda2 – 3'	CAGTCTGCCCTGACTCAGCCTGC	61~~60~~
Hu Vlambda3 – 3'	TCCTATGTGCTGACTCAGCCACC	62~~61~~

Primer name	Primer Sequence	SEQ ID NO:
Hu Vlambda3b – 3'	TCTTCTGAGCTGACTCAGGACCC	6362
Hu Vlambda4 – 3'	CACGTTATACTGACTCAACCGCC	6463
Hu Vlambda5 – 3'	CAGGCTGTGCTCACTCAGCCGTC	6564
Hu Vlambda6 – 3'	AATTTTATGCTGACTCAGCCCCA	6665

Please amend the section beginning on page 251, line 14, and continuing through line 24 as follows:

The PCR bands containing the VH domain and the VL domains can also be used to create full-length Ig expression vectors. VH and VL domains can be cloned into vectors containing the nucleotide sequences of a heavy (e. g., human IgGl or human IgG4) or light chain (human kappa or human lambda ~~ambda~~) constant regions such that a complete heavy or light chain molecule could be expressed from these vectors when transfected into an appropriate host cell. Further, when cloned heavy and light chains are both expressed in one cell line (from either one or two vectors), they can assemble into a complete functional antibody molecule that is secreted into the cell culture medium. Methods using polynucleotides encoding VH and VL antibody domains to generate expression vectors that encode complete antibody molecules are well known within the art.

Please amend the section beginning on page 253, line 11, and continuing through line 35 as follows:

Fusion protein constructs are created using polynucleotide sequences of the present invention with one or more copies (preferably at least 2, 3, 4, or more) of a conditional aggregation domain (CAD) a domain that interacts with itself in a ligand-reversible manner (i.e., in the presence of an inducing agent) using molecular biology methods known in the art and discussed elsewhere herein. The CAD domain may be the mutant domain isolated from the human FKBP12 (Phe^{36} to Met) protein (as disclosed in V.M. Rivera., et al., Science, 287:826-830, (2000), or alternatively other proteins having domains with similar ligand-reversible, self-aggregation properties. As a principle of design the fusion protein vector would contain a furin cleavage sequence operably linked between the polynucleotides of the present invention and the CAD domains. Such a cleavage site would enable the proteolytic cleavage of the CAD domains from the polypeptide of the present invention subsequent to secretion from the ER and upon entry into the trans-Golgi (J.B. Denault, et al., FEBS Lett., 379:113, (1996)). Alternatively, the skilled artisan would recognize that any proteolytic cleavage sequence could be substituted for the furin sequence provided the substituted sequence is

cleavable either endogenously (e.g., the furin sequence) or exogenously (e.g., post secretion, post purification, post production, etc.). The preferred sequence of each feature of the fusion protein construct, from the 5' to 3' direction with each feature being operably linked to the other, would be a promoter, signal sequence, "X" number of (CAD)x domains, the furin sequence (or other proteolytic sequence), and the coding sequence of the polypeptide of the present invention. The artisan would appreciate that the promoter ~~promotor~~ and signal sequence, independent from the other, could be either the endogenous promoter ~~promotor~~ or signal sequence of a polypeptide of the present invention, or alternatively, could be a heterologous signal sequence and promoter ~~promotor~~.

Please amend the section beginning on page 255, line 16, and continuing through line 26 as follows:

Moreover, it is unclear to what extent a glycosylation site in one species will be recognized by another species glycosylation machinery. Due to the importance of glycosylation in protein metabolism, particularly the secretion and/or expression of the protein, whether a glycosylation signal is recognized may profoundly determine a proteins ability to be expressed, either endogenously or recombinantly ~~recombinately~~, in another organism (i.e., expressing a human protein in E.coli, yeast, or viral organisms; or an E.coli, yeast, or viral protein in human, etc.). Thus, it may be desirable to add, delete, or modify a glycosylation site, and possibly add a glycosylation site of one species to a protein of another species to improve the proteins functional, bioprocess purification, and/or structural characteristics (e.g., a polypeptide of the present invention).

Please amend the section beginning on page 259, line 13, and continuing through page 260, line 6 as follows:

Once the DNA substrate has been purified, it would be subjected to Dnase I digestion. About 2-4μ~~u~~g of the DNA substrate(s) would be digested with .0015 units of Dnase I (Sigma) per μ~~u~~l in 100μ~~u~~l of 50mM Tris-HCL, pH 7.4/1mM MgCl2 for 10-20 min. at room temperature. The resulting fragments of 10-50bp could then be purified by running them through a 2% low-melting point agarose gel by electrophoresis onto DE81 ion-exchange paper (Whatmann) or could be purified using Microcon concentrators (Amicon) of the appropriate molecular weight cutoff, or could use oligonucleotide purification columns (Qiagen), in addition to other methods known in the art. If using DE81 ion-exchange paper, the 10-50bp fragments could be eluted from said paper using 1M NaCl, followed by ethanol precipitation.

The resulting purified fragments would then be subjected to a PCR assembly reaction by re-suspension in a PCR mixture containing: 2mM of each dNTP, 2.2mM MgCl2, 50 mM KCl, 10mM TrisoHCL, pH 9.0, and 0.1% Triton X-100, at a final fragment concentration of 10-30ng/μl. No primers are added at this point. *Taq* DNA polymerase (Promega) would be used at 2.5 units per 100μl of reaction mixture. A PCR program of 94 C for 60s; 94 C for 30s, 50-55 C for 30s, and 72 C for 30s using 30-45 cycles, followed by 72 C for 5min using an MJ Research (Cambridge, MA) PTC-150 thermocycler. After the assembly reaction is completed, a 1:40 dilution of the resulting primerless product would then be introduced into a PCR mixture (using the same buffer mixture used for the assembly reaction) containing 0.8μm of each primer and subjecting this mixture to 15 cycles of PCR (using 94 C for 30s, 50 C for 30s, and 72 C for 30s). The referred primers would be primers corresponding to the nucleic acid sequences of the polynucleotide(s) utilized in the shuffling reaction. Said primers could consist of modified nucleic acid base pairs using methods known in the art and referred to else where herein, or could contain additional sequences (i.e., for adding restriction sites, mutating specific base-pairs, etc.).

Please amend the section beginning on page 263, line 12, and continuing through line 30, including the Table presented therein as follows:

Briefly, using the isolated cDNA clone encoding the full-length K+betaM2 polypeptide sequence (as described in Example 9, for example), appropriate primers of about 15-25 nucleotides derived from the desired 5' and 3' positions of SEQ ID NO:1 may be designed to PCR amplify, and subsequently clone, the intended N- and/or C-terminal deletion mutant. Such primers could comprise, for example, an initiation ~~inititation~~ and stop codon for the 5' and 3' primer, respectively. Such primers may also comprise restriction sites to facilitate cloning of the deletion mutant post amplification. Moreover, the primers may comprise additional sequences, such as, for example, flag-tag sequences, kozac sequences, or other sequences discussed and/or referenced herein.

For example, in the case of the P24 to L428 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'-GCAGCA GCGGCCGC CCTGAGGTGGTAGAGCTGAATGTCG –3' (SEQ ID NO:2726) ***NotI***
3' Primer	5'- GCAGCA GTCGAC TAGATGATACTTCCTTAAAAGTTC -3' (SEQ ID NO:2827) ***SalI***

For example, in the case of the M1 to D263 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

Please amend the section beginning on page 264, line 5, and continuing through line 12 as follows:

5' Primer	5'- GCAGCA GCGGCCGC ATGGCTCTGAGTGGAAACTGTAGTC –3' (SEQ ID NO:2928) ***NotI***
3' Primer	5'- GCAGCA GTCGAC TGTATATTGGTTGATGAAAGATGCT -3' (SEQ ID NO:3029) ***SalI***

Representative PCR amplification conditions are provided below, although the skilled artisan would appreciate that other conditions may be required for efficient amplification. A 100 µl PCR reaction mixture may be prepared using 10ng of the template DNA (cDNA clone of K+betaM2), 200 µM 4dNTPs, 1µM primers, 0.25U Taq DNA polymerase (PE), and standard Taq DNA polymerase buffer. Typical PCR cycling condition are as follows:

Please amend the section beginning on page 266, line 9, and continuing through line 13 as follows:

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SEQUITHERM POLYMERASE™ ~~SequiTherm Polymerase~~. (Epicentre Technologies, Madison, Wisconsin). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

Please amend the section beginning on page 267, line 12, and continuing through line 32 as follows:

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 µg/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described elsewhere herein. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the

polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 µl of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 µl of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Please amend the section beginning on page 268, line 16, and continuing through line 26 as follows:

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1 µg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Please amend the section beginning on page 273, line 17, and continuing through page 274, line 36, as follows:

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR{ (zidovudine/AZT), VIDEX{ (didanosine/ddI), HIVID{ (zalcitabine/ddC), ZERIT{ (stavudine/d4T), EPIVIR{ (lamivudine/3TC), and COMBIVIR{ (zidovudine/lamivudine). Non-

nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE((nevirapine), RESCRIPTOR((delavirdine), and SUSTIVA((efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN((indinavir), NORVIR((ritonavir), INVIRASE((saquinavir), and VIRACEPT((nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE(, DAPSONE(, PENTAMIDINE(, ATOVAQUONE(, ISONIAZID(, RIFAMPIN(, PYRAZINAMIDE(, ETHAMBUTOL(, RIFABUTIN(, CLARITHROMYCIN(, AZITHROMYCIN(, GANCICLOVIR(, FOSCARNET(, CIDOFOVIR(, FLUCONAZOLE(, ITRACONAZOLE(, KETOCONAZOLE(, ACYCLOVIR(, FAMCICOLVIR(, PYRIMETHAMINE(, LEUCOVORIN(, NEUPOGEN((filgrastim/G-CSF), and LEUKINE((sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE(, DAPSONE(, PENTAMIDINE(, and/or ATOVAQUONE(to prophylactically treat or prevent an opportunistic Pneumocystis carinii pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID(, RIFAMPIN(, PYRAZINAMIDE(, and/or ETHAMBUTOL(to prophylactically treat or prevent an opportunistic Mycobacterium avium complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN(, CLARITHROMYCIN(, and/or AZITHROMYCIN(to prophylactically treat or prevent an opportunistic Mycobacterium tuberculosis infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR(, FOSCARNET(, and/or CIDOFOVIR(to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE(, ITRACONAZOLE(, and/or KETOCONAZOLE(to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR(and/or FAMCICOLVIR(to

prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE(and/or LEUCOVORIN(to prophylactically treat or prevent an opportunistic Toxoplasma gondii infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN(and/or NEUPOGEN(to prophylactically treat or prevent an opportunistic bacterial infection.

Please amend the section beginning on page 275, line 22, and continuing through page 276, line 5, as follows:

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE(~~(OKT3)~~muromonab-CD3), SANDIMMUNE(/NEORAL(/SANGDYA((cyclosporin), PROGRAF((tacrolimus), CELLCEPT((mycophenolate), Azathioprine, glucorticosteroids, and RAPAMUNE((sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR(, IVEEGAM(, SANDOGLOBULIN(, GAMMAGARD S/D(, and GAMIMUNE(. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

Please amend the section beginning on page 276, line 33, and continuing throuhg page 277, line 6, as follows:

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with RITUXIMAB ~~Rituximab~~. In a further embodiment, Therapeutics of the invention are administered with RITUXIMAB ~~Rituxmab~~ and CHOP, or RITUXIMAB ~~Rituxmab~~ and any combination of the components of CHOP.

Please amend the section beginning on page 277, line 15, and continuing through page 278 line 8, as follows:

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth ~~Gorwth~~ Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE((SARGRAMOSTIM() and NEUPOGEN((FILGRASTIM().

Please amend the section beginning on page 279, line 30, and continuing through line 33 as follows:

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 µ~~u~~g/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided herein.

Please amend the section beginning on page 293, line 11, and continuing through line 22 as follows:

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 μg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 108 TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 μg/ml ampicillin and 50 μg/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

Please amend the section beginning on page 295, line 33, and continuing through page 296, line 7, as follows:

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 10μg/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150μl. Proliferation or inhibition is quantitated by a 20h pulse (1μCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

Please amend the section beginning on page 296, line 32, and continuing through page 297, line 17, as follows:

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of 3H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 (l/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1 (g/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10^4/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of polypeptides of the invention (total volume 200 μl). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min.

at 1000 rpm and 100 (l of supernatant is removed and stored –20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 µul of medium containing 0.5 µuCi of 3H-thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of 3H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of polypeptides of the invention.

Please amend the section beginning on page 305, line 6, and continuing through line 23 as follows:

Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 µum (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 µul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber, 2.5 x 105 cells suspended in 50 µul M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO2 to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (~~Diff-Quick~~ DIFF-QUICK™, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

Please amend the section beginning on page 306, line 30, and continuing through line 31 as follows:

Example 44 - Effect of Polypeptides ~~Polypepides~~ of the Invention on Cord Formation in Angiogenesis

Please amend the section beginning on page 309, line 6, and continuing through line 31 as follows:

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita et al., Am J. Pathol 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita et al., ~~Takeshitaet al.~~ Am J. Pathol 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen et al. Hum Gene Ther. 4:749-758 (1993); Leclerc et al. J. Clin. Invest. 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number m the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

Please amend the section beginning on page 312, line 19, and continuing through line 20 as follows:

d) Positioning a pellet, containing 50ng- 5~~u~~μg of a polypeptide of the invention, within the pocket.

Please amend the section beginning on page 321, line 11, continuing through page 322, line 6 as follows:

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 µl of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 µl volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca^{2+}[[++]] and Mg^{2+}[[++]]) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 (100) > 10-0.5 > 10-1 > 10-1.5. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Please amend the section beginning on page 322, line 15, and continuing through line 27 as follows:

To search the Drosophila orthologue of human K+betaM2 gene, the K+betaM2 protein sequence (SEQ ID NO:2) was searched against the public Drosophila protein and genomic sequence

database from GenBank, using the BLAST software(1). A Drosophila gene CG10465 (SEQ ID NO:6768; Genbank Accession No: 17946205) was found to have the significant homology with the human K+betaM2 gene, with 23.7% identity (32.7% similarity) at the amino acid level covering the majority of the gene. The results of this preliminary database search suggest that CG10465 may be the putative Drosophila orthologue of the human K+betaM2 gene. In addition, the CG10465 protein has significant homolog to all K+channel tetramerisation domain proteins, as evidenced by its identity to the K_tetra PF02214 K+ channel tetramerisation domain (SEQ ID NO:74) Pfam hidden marup model (shown below). Due to the significant level of homology between the proteins, studies with CG10465 were undertaken in a Drosophila cell-based model for immunity.

Please amend the section beginning on page 323, line 5, and continuing through line 18 as follows:

Mammals have a complex immune response that relies on innate and adaptive immune pathways, and these pathways share similar classes of molecules. Most components of innate immunity are evolutionarily conserved from Drosophila to man, while only higher eukaryotes have acquired immunity (Silverman and Maniatis, 2001). Insects have a potent and rapid response to a broad spectrum of pathogens. Fungal and bacterial infections of Drosophila lead to transcriptional activation of antimicrobial peptide (AMP) genes. Induction of each AMP gene is regulated by a balance of inputs that are manifested by combinations of the three Rel/ NF-κB proteins- Relish, Dorsal and Dif. The AMP AttacinD gene is regulated by activation of Relish homodimers or heterodimers of Relish and Dorsal (Han and Ip, 1999). Activation of Rel/ NF-κB pathways are essential for the Drosophila innate immune response. For example, Drosophila mutations in the Relish gene do not express certain classes of antimicrobial peptides and are susceptible ~~suspectible~~ to gram-negative bacterial infection (Hedengren et al., 1999)

Please amend the section beginning on page 323, line 27, and continuing through line 36 as follows:

The experiments described herein correlate the function of the CG10465 protein to the regulation of the Drosophila innate immune response. Central to these studies was the generation of a "knock out" phenotype with double-stranded RNA-mediated interference (RNAi) of CG10465 mRNA in Drosophila Schneider 2 (S2) cultured cells. RNAi technologies were developed to produce sustained post-transcriptional gene-silencing and have been reported to work in S2 cells (Caplen et

al., 2000; Clemens et al., 2000). S2 cells can be induced by the bacterial cell wall component lipopolysaccharide ~~lipoploysaceride~~ (LPS) to express a subset of antimicrobial peptides.including attacin (Han and Ip, 1999). Experiments presented here test CG10465 RNAi in a LPS-inducible luciferase reporter system in S2 cells.

Please amend the section beginning on page 324, line 7, and continuing through page 325, line 20, as follows:

A stable S2 cell line was generated with an LPS-responsive AttacinD promoter fused to a luciferase reporter. S2 cells were purchased from InVitrogen and maintained at 25° C in complete 1x Schneider's Drosophila medium (Cat. No. 11720-034, Invitrogen, former GIBCO BRL) supplemented with 10% heat-inactivated fetal bovine serum (Cat. No. 10100-147, Invitrogen, former GIBCO BRL), 100 units/ml of penicillin, 100 µ~~u~~g/ml of streptomycin (100 X stock of Penicillin-Streptomycin, cat. No. 15140-148, from Invitrogen, former GIBCO BRL) and 20 mM L-Glutamine (100 x L-Glutamine, cat.No. 25030-149, from Invitrogen, former GIBCO BRL). A 1.6 Kb promoter region of the attacinD AMP gene was isolated from S2 genomic DNA by PCR using the primer pair: ATGAGGCTTGGATCAGCTTT (SEQ ID NO:68) (forward, 157904-157923bp of AE003718 Drosophila Genome project) and CCTGAAGCCTGACATTCCAT (SEQ ID NO:69) (reversed, 159547-159566bp of AE003718). Primers were obtained from GIBCOBRL . PCR condition : 96°C 4min, 94°C 2 min, 55°C 45 seconds, 72°C 2min, PCR 35 cycles. The 1.6kb attacinD PCR fragment was subcloned into a pCR2.1-TOPO vector (TOPO TA Cloning Kits, cat. No. K4500-01, Invitrogen). The attacinD promoter was subcloned from pCR2.1-TOPO vector into pGL3-Enhancer luciferase vector with restriction enzyme Sac I and Xho I(pGL3-Enhancer luciferase reporter vector, cat.no. E1771, Promega). A similar region was shown to be LPS responsive in a reporter assay (Tauszig et al., 2000). A final transfection construct, pGL3-enhancer- attacinD, was cotransfected with calcium phosphate methods with pCoHYGRO plasmid providing the hygromycin-B resistant gene as a stable selection, were used to transfect S2 cells (Inducible DES Kit, cat. No. K4120-01, Drosophila Expression System Instruction Manual,p16 from Invitrogen).

Briefly 19 µg ~~ug~~ of pGL3-enhancer- attacinD DNA was mixed with 1 µ~~u~~g of pCoHYGRO DNA and transfection buffer were used to transfect 6-12 X 10^6 cells/3 mls/well in 6-well Falcon tissue culture plate. Stable cells were selected and maintained in complete Schneider's medium containing 300µ~~u~~g/ml Hygromycin-B (Cat. R220-05, Invitrogen). Stable lines were tested for responsiveness to LPS (Han and Ip, 1999). Cells were treated with 20 µ~~u~~g/ml LPS (Cat. No L-2654,

Sigma) for 5 hours. Expression of luciferase was assayed with BRIGHT-GLO™ ~~Bright-Glo™~~ Luciferase Assay System (cat. No. E2620, Promega) and the luminescence signal was detected by 1450 MICROBETA™ Wallac Jet Liquid Scintillation & Luminesence Counter (Perkin Elmer Life Sciences).Two stable AttD-luc reporter cell lines (E4-1 and E4-9) were obtained after three rounds of limiting dilution and used for further studies.

Please amend the section beginning on page 326, line 5, and continuing through line 15 as follows:

5-15 µ~~u~~l cells in 100µ~~u~~l total volume were used for the luciferase assay, and 30-35µ~~u~~l cells in 100µ~~u~~l total volume were used for the proliferation assay. Luciferase assay plates were incubated for 5 hours after adding LPS at 20µ~~u~~g/ml. Proliferation assay plates were incubated for 2-3 hours before reading 490nm Optical Density. (CellTiter 96 Aqueous One Solution Cell Proliferation Assay from Promega, Cat. No. G3580).

Results presented below represent results of one experiment with E4-1 cells averaged in duplicate relative to control samples. Changes are relative to 1 depict fold change in luciferase expression ~~experssion~~ after normalization with cell number obtained in the proliferation assay. Similar results were obtained in 4 separate experiments and with the E4-9 stable cell line. NS is nonstimulated, LPS represents LPS treatment as described above.

Please amend the section beginning on page 327, line 13, and continuing through line 22 as follows:

Alternatively, under certain circumstances, antagonists of K+betaM2 could activate innate immunity. Likewise ~~Likeweise~~, antagonists of K+betaM2 could enhance the innate immune response and provide protection from invading pathogens in humans. In contrast, agonists of K+betaM2 would be expected to inhibit the NF-kB pathway and attenuate an inflammatory response, Hence, agonists of K+betaM2 may be useful in the treatment of inflammatory diseases including rheumatoid arthritis, asthma, multiple sclerosis, osteoarthritis, among others. K+betaM2 may also stimulate an immune response to tumors. In contrast, agonists of K+betaM2 could be useful for treating T-cell mediated autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, psoriasis, among others.

Please replace page 27 as filed with the replacement page 27 submitted herewith. The replacement page 27 is substantively an identical copy of the page as filed and thus does not introduce any new matter.

I. Status of the Claims

Claims 1-25 are pending the application. Claims 5-7, 10-15 and 20-25 have been withdrawn from consideration. Claims 1-4, 8, 9 and 16-19 have been examined.

The Patent Office objected to various aspects of the Specification, namely several misspellings, the inclusion of browser-executable code and the presence of unreadable text on page 27. The Patent Office has also objected to the presence of an amino acid sequence that does not have an associated SEQ ID NO.

The Patent Office objected to several claims. More particularly, the Patent Office objected to claims 1 and 16 as reciting non-elected subject matter. The Patent Office objected to claims 8 and 9 as depending from a non-elected claim. The Patent Office objected to claim 4 as reciting the plural "sequences" instead of the singular "sequence."

Claims 1-4, 8, 9 and 16-19 stand rejected under 35 U.S.C. §101 as not being supported by either a well asserted utility or a well established utility.

Claims 1-4, 8, 9 and 16-19 stand rejected under 35 U.S.C. §112, first paragraph, as not supported by either a well established utility or a well established utility and consequently, the Patent Office states, one of ordinary skill in the art would not know how to use the claimed invention.

Claims 1-4 and 16 stand rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which is not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention.

Claim 1 stands rejected under 35 U.S.C. §112, first paragraph, as conatining subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Lastly, claim 1 stands rejected under 35 U.S.C. §112, second paragraph, as indefinite.

II. Response to the Objections to the Specification

The Patent Office objected to the Specification for various reasons, which are presented in the Office Action on page 2, paragraph 3. These reasons include several inadvertent misspellings and typographical errors. The Patent Office also states that page 27 contains unreadable text.

Applicants have amended the Specification to correct these and other inadvertent misspellings and typographical errors applicants identified. Additionally, applicants are submitting herewith a replacement copy of page 27.

Next, the Patent Office objected to the disclosure because it contains an embedded hyperlink. Applicants have deleted all browser-executable code from the Specification.

Lastly, the Patent Office notes that the trademark RAPAMUNE, as well as others, appears on pages 273-275. The Patent Office further notes that this and other trademarks should be capitalized wherever it appears and should be accompanied by the generic terminology. Applicants have reviewed the Specification and believe that the recitation of the trademark RAPAMUNE, as well as other trademarks recited in the Specification, is accompanied by its generic name, where appropriate.

The amendments to Specification correct only formal matters and therefore do not present any new matter.

III. Response to the Objection Under the Sequence Rules

The Patent Office objected to the Specification as not complying with the rules governing Sequence Listings. The Patent Office notes the Specification discloses an amino acid sequence on page 322; applicants assume the Patent Office's objection is that one sequence appears on this page that is not associated with a SEQ ID NO.

Applicants submit herewith a substitute Sequence Listing that incorporates the K_tetra PF02214 K+ channel tetramerisation domain amino acid sequence appearing on page 322. Please replace the previously-filed Sequence Listing with the revised Sequence Listing submitted herewith. The replacement Sequence Listing is presented as a hard copy and as a CRF; a statement that the two versions are the same is also submitted herewith. Since the replacement Sequence Listing incorporates a sequence already presented in the Specification, the replacement Sequence Listing does not include any new matter.

Applicants note that the CG10465 protein presented in the alignment has been assigned SEQ ID NO:67 and is referenced as such in the preceding paragraph (see page 322, line 18).

IV. Response to the Claim Objections

The Patent Office objected to Claims 1 and 16 as reciting non-elected subject matter. The Patent Office objected to claims 8 and 9 as depending from a non-elected claim. The Patent Office objected to claim 4 because the Patent Office states the term "sequences" should be replaced with the singular "sequence."

Applicants have removed the recitation of "a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:1" from claim 1 and the recitation of "a

polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:41" from claim 16. Applicants believe these claims, as amended, do not recite any non-elected subject matter.

Claim 8 has been amended to depend from claim 1 rather than canceled claim 5. Claim 9 depends from claim 8, which depends from claim 1.

Claim 4 has been amended to replace the plural term "sequences" with the singular term "sequence."

These amendments to the claims are directed to formal issues and therefore do not embody any new matter.

V. Response to the Rejection of Claims 1-4, 8, 9 and 16-19 Under 35 U.S.C. §101

The Patent Office rejected claims 1-4, 6-8 and 16-19 under 35 U.S.C. §101 "because the claimed invention is not supported by either a well asserted utility or a well established utility." Office Action, page 4. Applicants traverse the rejection and submit the following comments.

Initially, applicants note that with regard to utility, the CAFC held "[A] 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' is sufficient." Fujikawa v Wattanasin, 93 F.3d 1559, 1565, 39 U.S.P.Q.2d 1895, 1900 (Fed. Cir. 1996). Bearing the above precedent in mind, the Patent Office's rejection of several of applicants' asserted utilities under 35 U.S.C. §101 will be addressed individually in the following paragraphs.

V.A. The Nucleic Acid Molecule (SEQ ID NO:1) Encodes a K+βM2 (Potassium Channel) Polypeptide

The Patent Office considers applicants' assertion that the claimed sequence is a potassium channel to be credible, but neither substantial nor specific. Applicants disagree for the following reasons.

The Patent Office contends applicants' characterization is not specific because the polypeptide is expressed in a "wide range of tissues." Applicants note that the expanded tissue profile presented in Figure 3 indicates that the polypeptide is predominantly expressed brain, testis and pancreas. Figure 5 indicates the various tissues of the brain in which the claimed sequence is expressed. Thus, applicants respectfully submit that a specific conclusion can, in fact, be drawn regarding the expression of the claimed sequences: a review of the expression profile indicates that the polypeptide is predominantly expressed in brain, testis and pancreas. To the extent that the other

tissues that express the claimed sequence do so, it is to a much significantly lesser degree. Applicants are therefore of the position that this asserted utility is specific.

Next, the Patent Office contends applicants' assertion is not substantial because "significant further research would be required of the skilled artisan to determine what SEQ ID NO:1's properties are." Office Action, page 6. Applicants respectfully disagree. Applicants have disclosed that the claimed sequence is an ion channel polypeptide expressed as shown in Figure 3. Thus, one of ordinary skill in the art would recognize that any properties of the claimed sequence not already explicitly disclosed in the instant Specification would be similar to the properties of other potassium channels, such as the ability to facilitate the transmission of potassium ions, the ability to modulate redox, etc. In other words, applicants submit that since the claimed sequence shares a degree of identity/homology with known potassium channels, it would also share similar properties.

V.B. The Nucleic Acid Molecule (SEQ ID NO:1) Encodes a Polypeptide That has K+βM2 (Potassium Channel) Activity

Generally, it is the Patent Office's position that applicants have not established that the claimed sequences encode a novel potassium channel beta subunit, particularly because the patent Office contends the Specification does not present any evidence to clearly suggest a specific biological activity for SEQ ID NO:1. Applicants respectfully disagree.

In support of its rejection under 35 U.S.C. §101, the Patent Office again presents arguments to the effect that applicants' conclusion regarding the identity of the claimed sequence as a novel potassium channel subunit is inaccurate and/or unsupported. Particularly, the Patent Office cites several references in support of its position that applicants' characterization of the claimed sequence by homology is untenable. Applicants submit that the homology data and the other data provided in the specification support applicants' characterization of the claimed sequence.

Continuing, applicants reiterate their arguments presented herein. Summarily, applicants contend that one of ordinary skill in the art, even in view of the cited references, would conclude the claimed sequence is a novel potassium channel subunit, as applicants have characterized the claimed sequence. Applicants again submit the identity between the claimed sequence and the human potassium channel K+Hnov28 supports applicants' contention. Applicants submit that the additional homology data provided in the specification further supports applicants characterization of the claimed sequences human potassium channel K+Hnov27 protein, human potassium channel K+Hnov27 protein and, as well as for the *C. elegans* and *Drosophila* sequences. Applicants are of

the position that the homology data and the other data presented in the Specification sufficiently support applicants' contention regarding the activity of the claimed sequence.

The Patent Office also states that applicants' assertion is not substantial because significant further research would be required to determine the properties of the claimed sequence. Applicants again submit that one of ordinary skill in the art would recognize that any properties of the claimed sequence not already explicitly disclosed in the instant Specification would be similar to the properties of other potassium channels, such as the ability to facilitate the transmission of potassium ions, the ability to facilitate a current, etc. Applicants are of the position that at least the homology of the claimed sequence with known sequences adequately supports applicants' contention regarding the activity of the claimed sequence.

V.C. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used to Make Polypeptides for Analysis, Characterization or Therapeutic Uses

The Patent Office states applicants' asserted utility is neither specific nor substantial. The Patent Office argues the Specification "does not disclose any known function for the claimed polypeptide or any disease state, toxin or poison associated with SEQ ID NO:1." Office Action, page 7. Applicants traverse the rejection and submit the following comments.

Applicants direct attention to page 21, lines 19 through 24 of the Specification, wherein applicants state: "[a] number of naturally occurring toxins are known to block potassium channels including apamin, iberiotoxin, charybdotoxin, margatoxin, noxiustoxin, kaliotoxin, dendrotoxin(s), mast cell degranuating (MCD) peptide, and beta.-bungarotoxin (.beta.-BTX). The K+betaM2 polypeptides may be used in in vitro and in vivo models to test the specificity of novel compounds, and of analogs and derivatives of compounds known to act on potassium channels." Thus, the Specification does, in fact, disclose toxins known to block potassium channels, such as the claimed sequence. Applicants also direct attention to page 22, line 16, through page 23, line 36, of the specification, wherein applicants present an extensive discussion of various diseases of testis and brain that may be diagnosed, prognosed, and/or prevented that may be related to the expression of the claimed K+βM2 sequence. The above specific diseases are only representative, and additional diseases and conditions in which the claimed sequence may play a role are provided in the Specification.

Applicants further direct attention to Example 56 (pages 322-328), wherein applicants demonstrate an association of the claimed sequence with the NFκB pathway. This was

accomplished by identifying a *Drosophila* orthologue of the claimed sequence, knocking out the identified orthologue and determining the effect of the knockout using RNAi techniques. The results of these experiments indicate that the *Drosophila* orthologue is involved in the regulation of the *Drosophila* innate immune response. Based on the degree of identity/similarity between the *Drosophila* orthologue and the claimed K+βM2 sequence, the claimed sequence is likely to have a function is the modulation of one or more mammalian immune pathways. In view of these results, applicants submit, therefore, that the Specification provides yet further specific diseases and conditions (e.g., immune pathway diseases and conditions) associated with altered levels of the claimed sequences.

Applicants also assert that the recited utility is substantial. Applicants submit that this asserted utility is presented in a form in which it can be employed in a real-world sense. The Specification provides guidance in this regard by indicating various conditions in which modulation of the expression of the claimed sequence can be employed to diagnose, prognose and/or prevent, as well as guidance in how to achieve the desired results such as that provided in Examples 27 and 28, for example.

Applicants also disagree with the Patent Office's position that significant experimentation would be required to determine how to use the identified polynucleotide. Applicants submit that any such experimentation, if in fact any experimentation would be required, would be simply routine and is unnecessary to identify or confirm a "real world" context. Applicants submit that a "real world" context is already established and no further experimentation is required in this regard.

V.D. The Nucleic Acid Molecule (SEQ ID NO:1) Does Not Have a Known Ligand

The Patent Office argues that the Specification does not disclose any specific ligands for a polypeptide encoded by the claimed sequence and that one of ordinary skill in the art would have to employ significant experimentation to identify a disorder associated with the claimed sequence.

In response, applicants direct attention to Example 9 of the Specification, found on page 230. Example 9 provides instructions for identifying the cognate ligand of the claimed sequence, for example by employing an epitope tag. In view of the guidance provided in the specification, applicants submit that the asserted utilities would not require significant or undue experimentation, since guidance is provided as to how a ligand could be identified.

Further, applicants again note that an extensive list of diseases the may be associated with the claimed sequence is presented in the specification, at least at the points indicated herein above.

Applicants are therefore of the opinion that the asserted utility is specific, since it recites particular diseases, substantial, since guidance is provided for the identification of ligands.

V.E. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used in Drug Design

The Patent Office next argues that the use of the claimed sequence in drug design is credible, but not substantial or specific. The Patent Office contends the asserted utility is not specific because "it is not known what condition, diseases, or disorders would be the target of the designed drugs, nor is it substantial since significant further research would be required to determine how to use the identified potential drugs." Office Action, page 8. The Patent Office continues, "[i]t is not clear how the skilled artisan would use a potential drug identified by this method." Office Action, page 8. Applicants respectfully disagree.

Applicants again submit that a list of diseases and conditions that could be treated with a modulator (a drug) of the claimed sequence is disclosed in the Specification, as discussed above. Additionally, guidance is provided for the preparation of formulations comprising such a modulator that could be employed in the treatment of a disease in addition to a discussion of dosing procedures. Applicants therefore submit that the asserted utility is specific.

Additionally, in view of the discussion presented in the Specification regarding the preparation and administration of such a modulator, applicants are of the position that the asserted utility is also substantial, and that significant further research would not be required.

V.F. The Nucleic Acid Molecule (SEQ ID NO:1) is Useful as Probes or Primers

The Patent Office argues that the asserted utility of using the claimed sequence as a probe or a primer is credible but neither substantial nor specific. Applicants respectfully disagree.

Applicants again submit that the homology and *Drosophila* data presented in the Specification support applicants' contention that the claimed sequence is a K+βM2 is a potassiuum channel β subunit polypeptide. As noted herein, the homology data involves polypeptides that are known to the potassium channels. Applicants are of the position that this observation, in conjunction with the *Drosophila* data, would convince one of ordinary skill in the art of applicants' assertion. Again, as noted herein, applicants have supplied specific diseases in which the claimed sequence can be employed in the cited utility. Consequently, applicants are of the position that the asserted utility is substantial.

Applicants also believe the cited utility is specific since, although all nucleic acids can be used as probes or primers, not all nucleic acids can be used as primers or probes for the claimed sequence. Thus, applicants contend the asserted utility is specific, substantial and, as the Patent Office agrees, credible, thereby meeting the statutory requirement of utility.

V.G. The Nucleic Acid Molecule (SEQ ID NO:1) has Therapeutic Uses (Gene Therapy)

The Patent Office states applicants' asserted utility of gene therapy is not specific or substantial. Applicants disagree with the Patent Office's characterization of this asserted utility.

The Patent Office states "[t]he specification does not disclose any known disease state, toxin or poison associated with SEQ ID NO:1." Office Action, page 9. The Patent Office continues, "significant further research would be required to determine how to use the identified polynucleotide." Office Action, page 9. In response, applicants direct attention to the section of the Specification beginning on page 157 and entitled "Biological Activities," and continuing through the sections following the section entitled "Diseases at the Cellular Level" on page 178. In this section, applicants provide both general descriptions of types of diseases and conditions that may be treatable using the polynucleotides of the present invention (e.g., in a gene therapy application) as well as specific diseases and conditions that may be treatable. Support for association of the claimed sequences with these and other conditions is presented in the Examples and Drawings of the instant application. Thus, applicants submit that the specification describes specific conditions that may be treatable using the polynucleotide sequences of the present invention.

V.H. The Nucleic Acid Molecule (SEQ ID NO:1) is Useful in Screening Assays (Microchip, PCR-based, Yeast-based, RFLP)

The Patent Office states that the asserted utility of employing a sequence of the present invention as a component of a gene chip is not specific or substantial. Applicants respectfully disagree and submit the following comments.

As stated in the specification, "such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including proliferative diseases and conditions." (Specification, page 140, lines 29-33).

Applicants submit that the above representative discussion, coupled with applicants arguments in support of the characterization of the claimed sequences as encoding a K+betaM2 potassium channel modulating subunit, supports applicants assertion that the use of the claimed sequences as a component of a gene chip is specific and substantial.

Thus, applicants are of the belief that although a gene chip can be made for any polynucleotide, the present specification discloses particular sequences that can be attached to a gene chip, namely K+βM2 sequences, and fragments thereof, disclosed. As indicated, such a gene chip could be employed in screening for polymorphisms, for example those associated with a disease state or other undesirable condition. Applicants therefore submit that the asserted utility is in fact substantial, credible and specific.

V.I. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used to Make Antisense and/or Sense Methodology, Ribozymes and Peptide Nucleic Acids (PNAs)

The Patent Office contends this asserted utility is neither specific nor substantial. The Patent Office contends this utility is not substantial because the encoded polypeptide does not have a substantial utility. The Patent Office further states that the asserted utility is not specific, because any nucleic acid can be employed in the same applications. Applicants disagree and submit the following comments.

For the reasons discussed herein, it is applicants position that the claimed sequence has been characterized and identified to a degree that one of ordinary skill in the art would not question its identity or cellular role. Accordingly, applicants are of the position that the asserted utility is substantial. Further, although all nucleic acids can be used as probes or primers, not all nucleic acids can be used as antisense sequences for the claimed sequence. Thus, the asserted utility is specific, substantial and credible.

V.J. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used to Make Antigenic Peptides

With respect to the recited utility of producing antibodies, the Patent Office states: "If substantial further research is required to determine how to use the full-length polypeptide, then substantial further research is also required to determine how to use antibodies generated from antigenic fragments." Office Action, page 10.

Applicants respectfully disagree with the Patent Office's conclusion. Applicants submit that specific and substantial details regarding the claimed polynucleotides and polypeptides is provided

in the specification as filed. For example, tissue profiling was performed that localized expression of the claimed polypeptides to testis, brain and pancreas (see Figure 3). Expanded expression profiling (see Figure 54) confirmed high expression in various brain tissues. Further, RNAi data on protein CG10465, the putative *Drosophila* ortholog of the claimed human K+βM2, suggests that in *Drosophila* this protein regulates the LPS-response pathway (see Example 56).

This data provides specific and substantial details regarding the claimed polypeptides and polynucleotides of the present invention. Thus, contrary to the Patent Office's position, the specification does indeed provide specific and substantial details of the claimed polynucleotides and polypeptides of the present invention. Consequently, Applicants submit that the antigenic peptides and the associated antibodies of the present invention, as well as the polypeptides, have utility that is specific, substantial and credible.

V.K. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used to Make Chimeric (Fusion) Proteins

It is the Patent Office's position that applicants' asserted utility of making a fusion protein is not substantial or specific. The Patent Office states "it is not clear how the skilled artisan would use a chimeric polypeptide for therapeutic, diagnostic or research uses." Office Action, page 10. Applicants respectfully disagree.

Applicants direct attention to page 67, line 36, through page 69, line 16, wherein applicants describe the formation of a fusion protein that may increase the half-life of the protein *in vivo*. A fusion protein of the present invention can also offer advantages in purification, as described in the Specification. A detailed discussion of the advantages, uses and formation of a fusion protein of the present invention is presented starting on page 120, line 13, through page 124, line 8.

The Specification also states various specific uses for the fusion proteins of the present invention. For example, the Specification states "[m]oreover, polypeptides of the present invention may be useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein" (Specification, page 164, lines 33-36). The Specification further states "Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis." (Page 165, lines 12-14). The Specification also discloses a similar use for fusion proteins of the present invention in inhibiting the metastasis of proliferative cells or tissues (page 165, lines 29-30), and enhancing the

immunogenicity and/or antigenicity of proliferating cells or tissues (page 166, lines 13-18). Thus, applicants submit the use of the claimed sequences as fusion proteins provides a specific, substantial and credible utility, and that these fusion proteins can be employed in real world applications, such as those highlighted above.

V.L. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used to Transform Host Cells

It is the Patent Office's position that "the skilled artisan would have to experiment significantly in order to determine how the transformed host cells could be used." Office Action, page 10. Applicants again disagree.

The Specification presents an extensive discussion on the recombinant expression of the claimed sequences. Various applications for the expressed protein itself are also presented, including those discussed herein. Thus, applicants are of the position that the host cells could be used to generate recombinant protein for use in any of the applications described herein and in the Specification, thus making the use of host cells substantial. Applicants believe this use is also specific since, as discussed above, the asserted utilities are specific to the claimed K+βM2 sequence of the present invention.

V.M. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used in Chromosomal Mapping

Next, the Patent Office states the use of the claimed sequence in a chromosomal mapping protocol is not substantial or specific. More particularly, the Patent Office states, "further research would be required for the skilled artisan to determine where this particular sequence is mapped in order to use the nucleic acid molecule in the asserted utility as a chromosomal map probe." Office Action, page 11.

Applicants direct attention to Example 12, page 236, wherein specific guidance is provided for a chromosomal mapping protocol. Applicants also provide specific details regarding PCR conditions and types of gels that can be employed. Template DNA is commercially available and can be employed. Using the provided guidance, coupled with knowledge of the claimed sequence as provided in the Specification, applicants submit that one of ordinary skill in the art would not need to employ substantial further research in order to use the sequence as a chromosome map probe, even in view of the number of known mammalian potassium channels.

V.N. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used as Molecular Weight Markers on Gels

The Patent Office argues this asserted utility is neither substantial nor specific. The Patent Office submits that the specification does not disclose any unique attributes of the claimed sequence that would motivate one of ordinary skill in the art to use the sequence as suggested. Applicants disagree.

The specification discloses a range of diseases and conditions associated with potassium channels. Such diseases can be a function of the presence, absence, truncation, duplication or other modification of the claimed sequence. Thus, it would be desirable to determine the presence, absence or modification of the claimed sequence. This can be accomplished, for example, by purifying the sequence and determining the presence, absence or condition of this sequence using electrophoresis. Applicants further submit that significant further research would not be required to determine how to use the identified molecular weight markers because guidance is provided in the Specification on various uses of the claimed sequence that include diagnostic steps in which such markers could be employed.

V.O. The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used to Make Probes and Oligomers

The Patent Office argues that applicants' asserted utility of hybridization probes is not substantial or specific. It is the Patent Office's position that there is no substantial utility for the polypeptide and thus there is not substantial utility for the probes used to identify the claimed sequence in the sample. Applicants disagree with the Patent Office's analysis.

Applicants direct attention to page 39, lines 9-20, wherein applicants describe designing nucleic acid hybridization probes that will detect nucleic acid sequences comprising the claimed sequence and/or the cDNA contained in the deposited clone. The specification also provides that such probes can hybridize with nucleic acid molecules in biological samples and can be employed in a range of forensic and diagnostic methods. For instance the hybridization probes of the present invention can be employed as reagents in the detection and isolation of a claimed K+betaM2 nucleotide sequence from a tissue sample.

Such probes can also be employed in various applications. Additionally, applicants note the Patent Office's assertion that no nexus between a disease state and the claimed sequence is disclosed in the Specification. Applicants note, however, that the *Drosophila* data suggests a role for the claimed sequence, at least, in the LPS pathway and an association with the NF-kB pathway.

Applicants submit that, cumulatively, the data described in the Specification indicates a specific to the asserted utility, and that in view of the homology data and other data provided, the asserted utility is substantial.

V.P The Nucleic Acid Molecule (SEQ ID NO:1) Can be Used to Make Transgenic Animals

It is the Patent Office's position that applicants' asserted utility of generating a transgeneic animal is not specific or substantial. The Patent Office argues one of ordinary skill in the art would have to experiment to determine how to use a generated transgenic animal. Applicants again disagree.

Applicants reiterate the arguments presented herein above regarding the disclosure of the various general and specific disease states and conditions that are related to the sequences claimed in the present application. Summarily, applicants submit that the Specification discloses a range of diseases and conditions that may be treatable by employing (or removing) a polynucleotide sequence of the present invention.

Continuing, applicants direct attention to Example 32, found on page 287 of the Specification. In one aspect, this Example describes incorporating (i.e., "knocking in") a gene (e.g., a gene encoding a K+βM2 polypeptide) into some or all cells of an animal. Continuing, Example 33, found on page 289 of the Specification, describes removing (i.e., "knocking out") a gene (e.g., a gene encoding a K+βM2 polypeptide). The decision to knock in or knock out a gene can depend on the nature of the disease or condition being studied or treated. As discussed in the specification, in some situations it might be desirable to knock in a gene, for example, when expression of the gene is non-existent in the wild type animal. In other situations it might be desirable to knock out a gene, for example when high expression levels of the gene lead to a disease or condition. Examples of both situations are discussed in the Specification.

In view of the above, applicants submit that the generation of a transgenic animal is a substantial, credible and specific utility, since both specific disease states and a discussion of characteristics of the claimed sequence are presented.

Summarily, Applicants submit that each of the utilities provided in the specification, including those identified by the Patent Office, are specific, substantial and credible. Accordingly, applicants respectfully request that the rejection of claims 1-4, 8, 8 and 16-19 under 35 U.S.C. §101

be reconsidered and withdrawn. Applicants further submit that claims 1-4, 8, 8 and 16-19 are in condition for allowance and courteously solicit the same.

VI. Response to the Rejection of Claims 1-4, 8, 9 and 16-19 Under 35 U.S.C. §112, First Paragraph

The Patent Office rejected claims 1-4, 8, 9 and 16-19 under 35 U.S.C. §112, first paragraph for various enumerated reasons. Applicants respectfully traverse the rejection and submit the following comments, which, for clarity, are directed to the rejections in the order the Patent Office presented them. Before addressing the specific rejections, applicants submit the following comments.

With regard to the enablement requirement, applicants submit that, as a matter of Patent Office practice, the burden rests upon the Patent Office to establish a *prima facie* case of a failure to comply with 35 U.S.C. § 112, first paragraph, with respect to the invention described and claimed in applicants' presumptively enabling patent application. *In re Marzocchi*, 58 C.C.P.A. 1069, 439 F.2d 220, 169 U.S.P.Q. 367 (C.C.P.A. 1971), *In re Wertheim*, 541 F.2d 257, 263, 191 U.S.P.Q. 90, 97 (C.C.P.A. 1976). More specifically, the Patent Office bears the burden of establishing by a preponderance of evidence that one of ordinary skill in the art would not be enabled to practice the present invention after considering the present disclosure in combination with what is known in the art. It is applicants' position that in the present case, the Patent Office has not met its burden.

Applicants now respond to the specific rejections made by the Patent Office.

(1) First the Patent Office rejected claims 1-4, 8, 9 and 16-19 under 35 U.S.C. §112, first paragraph, as "not supported by either a well asserted utility or a well established utility for the reasons set forth above" (Office Action, page 13) leading the Patent Office to conclude that one of ordinary skill in the art would not be able to make and use the claimed invention.

For the reasons discussed above, applicants submit that the present invention is supported by at least one specific, substantial and credible utility. These utilities are discussed in detail in the Specification, thereby satisfying the requirements of 35 U.S.C. §112. Applicants submit that the specification of the present application teaches the manner in which to make and use the present invention; the Examples, for instance, provide explicit guidance in this regard. The present application describes how to make or obtain the claimed nucleic acids, vectors and host cells. In addition, the present application discloses how to use the isolated nucleic acids encoding K+βM2 and variants thereof, recombinant vectors and host cells containing the nucleic acid to make K+βM2

polypeptides, and to identify modulators thereof useful for the treatment of various disorders, notably immune system disorders.

(2) The Patent Office's next rejection of claims 1-4, 8, 8 and 16-19 under 35 U.S.C. §112, first paragraph, relates to applicants' recitation of the use of derivatives and fragments of SEQ ID NO:1 and is based on the Patent Office's position that "applicant has provided little or no guidance beyond the mere presentation of sequence data to enable one of ordinary skill in the art to determine, without undue experimentation, the positions in the protein which are tolerant to change (e.g., such as by amino acid substitutions or deletions), and the nature and extent of changes that can be made at these positions." Office Action, page 14.

In this regard, it appears that the Patent Office is requiring that applicants submit working examples in order to comply with the requirements of 35 U.S.C. §112, first paragraph. The applicable statute and case law, however, mandates no such requirement. While the presence or absence of working examples can be a consideration in the overall evaluation of enablement, working examples are not required under 35 U.S.C. §112, first paragraph, to comply with the enablement standard presented therein. Indeed, the M.P.E.P. states that a U.S. patent application need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *M.P.E.P. §2164.02.*

Turning next to the Patent Office's assessment of the amount of experimentation required to practice the claimed invention, it is applicants' position that, even if it might require experimentation to generate and/or use derivatives/fragments of a K+βM2 sequence, the quantity of experimentation to be performed by one skilled in the art is only one factor involved in determining whether "undue experimentation" is required to make and use the invention. "An extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." *In re Colianni*, 195 U.S.P.Q. 150, 153 (C.C.P.A. 1977). "The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the U.S. patent application in question provides a reasonable amount of guidance with respect to the direction in which experimentation should proceed." *In re Wands*, 8 U.S.P.Q.2d at 1404 (citing *In re Angstadt*, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976)).

The specification provides a nucleic acid sequence encoding the claimed K+βM2 polypeptide, as well as the sequence of the encoded polypeptide. Applicants submit that contrary to

the Patent Office's position, no experimentation is required, beyond the application of routine molecular biological techniques, in order to use the claimed polynucleotides to make variants and fragments useful for the purposes discussed in the Specification.

As noted above, the polypeptides and polynucleotides of the present invention have been described in the specification. This knowledge can be employed to make, study and use variants of the claimed polypeptides and polynucleotides. Indeed guidance is provided in the specification (see page 57, line 33 through page 62, line 18, including Table 3 presented therein, for example) in preparing such variants. Such variants can be employed in the same roles as the characterized claimed polypeptides and polynucleotides. For instance, a variant prepared in accordance with the present invention might exhibit an enhanced activity that might be desirable in a given application of the sequences of the present invention.

Applicants again note that the Patent Office's burden is to demonstrate that the disclosure, combined with what is known in the art, does not enable one of ordinary skill in the art to practice the invention commensurate with the scope of the claims. Given the high level of skill in the art, it is applicants' position that the Patent Office has not provided sufficient evidence that one of ordinary skill in the art would not be enabled by the present disclosure, combined with what is known in the art, to employ the compositions and methods of the present disclosure, particularly in view of the specific, substantial and credible utilities recited in the specification and the extensive discussion of derivatives and fragments presented in the Specification.

(3) The Patent Office has also rejected claims 1-4, 8, 9 and 16 "as containing subject matter which was not described in the specification in such as way as to reasonably convey to one skilled in the relevant art that the inventor(s) at the time the application was filed, had possession of the claimed invention." Office Action, page 15. It is the Patent Office's position that "distinguishing characteristics of the claimed genus are not described. The only adequately described species is a nucleic acid comprising SEQ ID NO:1." Office Action, page 16

Applicants note that the *Guidelines for Examination of Patent Applications Under the 35 USC 112, ¶1 "Written Description" Requirement* indicate that the written description requirement can be satisfied by "sufficient description of a representative number of species by actual reduction to practice, reduction to drawings or by disclosure of relevant, identifying characteristics." *Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1 "Written*

Description" Requirement, 66 Fed. Reg. 1099, 1105 (Jan. 5, 2001). See also, *University of California v. Eli Lilly*, 119 F.3d 1559, 43 U.S.P.Q.2d 1398 (Fed. Cir. 1997) and *MPEP* §2163.II.A.ii.

Applicants submit that the specification as filed discloses an extensive list of representative species and relevant characteristics of the species, thereby satisfying the written description requirement of 35 U.S.C. §112, first paragraph. Particularly, applicants direct attention to pages 29-33 of the specification, wherein an extensive list of N-terminal and C-terminal deletion polypeptides are disclosed. The specification states "polynucleotide sequences encoding these polypeptides are also provided" (Specification, page 31, line 27). These deletion polypeptides comprise fragments of the nucleic acid and polypeptide sequences of SEQ ID NO:1. These polynucleotide sequences (a) would be expected to hybridize under stringent conditions (which are described in the specification, including Table 2 presented therein) to the polynucleotides specified in the claims, (b) have a nucleotide sequence that is at least 99.8% identical to a sequence provided in the claims; and (c) do not encode the polypeptide set forth as SEQ ID NO:1. Therefore, applicants submit that the amino acid and/or polynucleotide sequences are representative of the claimed genus.

Applicants further note that in addition to the disclosed N- and C-terminal deletion polynucleotides, the specification describes the use of conservative substitutions in generating variants of the polynucleotides and polypeptides of the present invention (see, e.g., Table 3). Such variants can have a different sequence from that of SEQ ID NO:1, yet retain the properties recited in the claims. Thus, this group of variants highlights yet more species that are representative of the claimed genus.

Next, applicants draw attention to the statement that "[g]enerally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosures necessary to satisfy the written description requirement." *Guidelines for Examination of Patent Applications Under the 35 USC 112, ¶1 "Written Description" Requirement*, 66 Fed. Reg. 1099, 1105 (Jan. 5, 2001). As noted, applicants submit that the relative level of skill in the pertinent field is very high. Therefore, applicants submit that given the high level of skill in the field identified by the Patent Office, the large number of species representative of the claimed genus disclosed in the present specification, coupled with the discussion of their relevant identifying characteristics, the invention is fully described in accordance with 35 U.S.C. §112, first paragraph.

Applicants submit that, in view of (a) the extensive disclosure of N- and C-terminal deletion polypeptides and the polynucleotides that encode these polypeptides, presented in the specification, (b) the disclosure of species comprising conservative substitutions of SEQ ID NO:1, (c) the

recitation of identifying characteristics of the members of the claimed genus, and (d) the high level of skill in the art, claims 1-4, 8, 9 and 16 are in accord with the Written Description Guidelines and the pertinent case law (see, e.g., *University of California v. Eli Lilly*, 119 F.3d 1559, 43 U.S.P.Q.2d 1398 (Fed. Cir. 1997)), and that the written description requirement of 35 U.S.C. §112, first paragraph, has been met. Summarily, applicants submit that one of ordinary skill in the art would recognize that Applicants had invented what was claimed, which is the standard against which the adequacy of a written description is gauged. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 19 U.S.P.Q.2d 1111 (Fed. Cir. 1991). Accordingly, applicants respectfully request that the rejection of claims 1-4 and 16 under 35 U.S.C. §112, first paragraph, be reconsidered and withdrawn.

VII. Response to the Rejection of Claim 1 Under 35 U.S.C. §112, First Paragraph, as Related to the Recitation of an "Allelic Variant of SEQ ID NO:1"

The Patent Office rejected claim 1 under 35 U.S.C. §112, first paragraph, for reciting "an allelic variant of SEQ ID NO:1." It is the Patent Office's position that no chromosomal location is disclosed in the Specification and, therefore, it would require undue experimentation to practice the invention as claimed. Applicants respectfully disagree.

In response, applicants direct attention to Example 12, wherein applicants provide guidance on chromosome mapping. Applicants submit that one of ordinary skill in the art, employing the guidance provided in the Specification, notably Example 12, would readily be able to use the invention as claimed. Applicants note that routine experimentation is permissible and, given the guidance provided in the Specification and the high level of skill in the art, applicants submit that any experimentation a skilled artisan that might be required to practice the claimed invention would be routine. Applicants are of the position that if any experimentation were to be required, it would not be undue, given the clear guidance provided in the Specification. Accordingly, applicants respectfully request that the rejection of claim 1 under 35 U.S.C. §112, first paragraph, be reconsidered and withdrawn.

VIII. Response to the Rejection of Claim 1 Under 35 U.S.C. §112, First Paragraph, as Related to the ATCC Deposit

The Patent Office rejected claim 1 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the

invention. More particularly, the Patent Office states that although the claimed nucleic acid molecule has been deposited with the ATCC, as evidenced by its Deposit No., which is reported in the Specification, there is no indication in the Specification as to the public availability of the nucleic acid molecule.

Applicants submit herewith a statement by an attorney of record over his or her signature and registration number stating that the specific nucleic acid molecules have been deposited under the Budapest Treaty and that the nucleic acid molecules will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. In view of this submission, applicants respectfully request that the rejection of claim 1 under 35 U.S.C. §112, first paragraph, be reconsidered and withdrawn.

Applicants additionally note that the correct address of the ATCC appears in the Specification on page 13, lines 11-12.

IX. Response to the Rejection of Claim 1 Under 35 U.S.C. §112, Second Paragraph

The Patent Office rejected claim 1 under 35 U.S.C. §112, second paragraph, as indefinite. The Patent Office contends the term "stringency" is a relative term, which renders the claim indefinite. The Patent Office also argues that neither the claim nor the specification provides a standard for ascertaining the requisite degree and that one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Applicants traverse and submit the following comments.

Applicants direct attention to page 14, line 16 through line 20, wherein the term "stringent hybridization conditions" is defined. Applicants also direct attention to page 41, line 30, through page 45, line 28, including Table II presented therein, wherein applicants discuss "stringent hybridization conditions" and provide examples of such conditions.

Applicants submit that claim 1 is in full compliance with 35 U.S.C. §112, second paragraph. Accordingly, applicants respectfully request that the rejection of claim 1 under 35 U.S.C. §112, second paragraph be reconsidered and withdrawn.

X. Conclusions

In light of the above amendments and remarks, applicants respectfully request that the rejections of record be withdrawn. Applicants further submit that the subject patent application is in condition for allowance and courteously solicit a Notice of Allowance.

If any small matter should remain outstanding after the Patent Office has had an opportunity to review the instant paper, the Patent Office is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Office Action.

Although it is believed no additional fee is due, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment associated with the filing of this correspondence to Deposit Account Number 19-3880. Furthermore, if any extension of time not already accounted for is required, such extension is hereby petitioned for, and it is requested that any fee due for said extension be charged to Deposit Account Number 19-3880.

Respectfully submitted,

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